ORIGINAL RESEARCH

Correlations Between Serum Cytokine Levels and the Use of a Moisturizer in Elderly Women in Accordance with the Improvement of Objective and Subjective Skin Condition

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Purpose: In the skin of elderly people with dryness, the production of inflammatory cytokines tends to be induced under the influence of external stimuli. Therefore, there has been a hypothesis that the deterioration of skin conditions due to aging is linked to systemic inflammation. This study aimed to verify the possibility that the use of moisturizer improves skin condition and suppresses systemic inflammation.

Methods: As an open study, the participants (n=75) were randomly assigned to either control group or moisturizer group. Participants in the moisturizer group used a moisturizer called Grafa Moisture Keep Milk MC at least twice a day for four weeks on the entire body below the neck. Objective skin conditions (overall dry skin score, water content of the stratum corneum, and transepidermal water loss) and serum cytokine levels (IL-1α, IL-1β, IL-4, IL-5, IL-6, IL-8, and TNF-α) were evaluated before and after the study in both groups. Subjective skin condition (questionnaire evaluation) was also assessed in the moisturizer group after the study.

Results: Serum IL-6 level was significantly reduced in the moisturizer group (n=16) compared with the control group (n=36). In addition, there was an inverse correlation between serum IL-5 and the subjective moisturizing effect in the questionnaire evaluation, suggesting that the moisturizer improved subjective symptoms of dryness by reducing IL-5 levels. Furthermore, there was a positive correlation between IL-5 and IL-6, indicating that they are regulated by common upstream factors. A significant positive correlation of transepidermal water loss with serum IL-4 levels was also detected.

Conclusion: The application of the moisturizer to the entire body not only improved subjective and objective skin condition, it may also reduce the levels of circulating inflammatory cytokines.

Umin Clinical Trials Registry: Registration number: UMIN 000052024.

Keywords: aging, cytokine, dryness, inflammation, skin care

Introduction

Chronic inflammation in elderly people is believed to promote the development of age-related inflammatory diseases such as arteriosclerosis, diabetes, osteoporosis, and Alzheimer's disease.[1–3] Inflammation is also a major risk factor for morbidity and mortality from these diseases in elderly people.[1–3] For example, IL-1α, IL-1β, IL-6, and TNF-α promote plaque formation in arteriosclerosis, whereas IL-6 and TNF-α induce the formation of amyloid-β in Alzheimer's disease.[4,5] The cause of accumulation of chronic inflammation due to aging has been explored in various studies. Suppression of chronic inflammation in elderly people may prevent the onset and exacerbation of age-related diseases. However, the mechanism behind the accumulation of inflammation with aging, and how to suppress age-related chronic inflammation are unclear.[1–3]

In the skin of elderly people, water content of the stratum corneum and skin surface lipid content are lower than in the skin of younger people, which causes skin dryness.[6,7] The use of moisturizer is commonly recommended to improve the skin

dryness. Our previous study showed that using a moisturizer called Grafa Moisture Keep Milk MC for four weeks in xerosis patients led to improvements in dry skin, such as lower skin dryness scores and increased water content of the stratum corneum.[8] Moreover, in aged skin with impaired barrier function, the production of inflammatory cytokines is induced under the influence of external stimuli. In murine models, aged mice have been shown to have higher levels of inflammatory cytokines both in the skin and the sera than young mice.[9] Furthermore, it has been reported that applying a moisturizer reduces the levels of cutaneous and serum cytokines of aged mice to levels comparable to those of young mice.[9] Therefore, the use of moisturizers may not only improve dry skin in the elderly, but also prevent and improve age-related inflammatory diseases by suppressing systemic inflammation through improved skin condition. To test this hypothesis, we therefore examined changes in subjective or objective skin conditions and serum levels of inflammatory cytokines in elderly women by the use of a moisturizer for four weeks.

Materials and Methods

Participants

The participants were 75 Japanese women aged between 70 and 90 who had no history of severe illness or complications requiring treatments. To ensure fairness, the participants were recruited in a third-party institution (Hakodate Central General Hospital). Approval was obtained from the Hakodate Central General Hospital Medical Ethics Committee (approval numbers: 2021–25) in accordance with the Declaration of Helsinki and ethical guidelines for clinical research. This study was conducted between January and June 2022, and was initiated after fully explaining the study contents to the participants and obtaining their written informed consent.

Test Product

Grafa Moisture Keep Milk MC (Grafa Laboratories Co., Ltd., Osaka, Japan) was used as the moisturizer in this study. This test product is characterized by the formulation of partially myristoylated carboxymethyl chitosan (PMCMC), a polymer compound expected to have strong moisturizing power due to its high water retention capacity exceeding that of hyaluronic acid and strong film-forming ability due to its high affinity with the stratum corneum.[10]

Test Protocol

As an open study, the participants were randomly assigned to a control group (n=36) or a moisturizer group (n=36) by 1:1 using the substitution block method (Figure 1). The number of subjects was determined based on another previous study (33 elderly people with emollients and 30 without).[11] The participants in the moisturizer group were instructed to use the test product at least twice a day for four weeks on the test area (the entire body below the neck). Meanwhile, previous surveys reported that elderly people do not have the habit of using moisturizer to their bodies,[12,13] we decided that the control group consisted of subjects who did not apply moisturizers to the test areas (arms, legs, front of the body, and back) during the test period. Participants in both groups were allowed to use moisturizers other than the test product on the non-test area.

Three participants in the control group withdrew their consent for personal reasons (eg schedule or moving) before completing the test, so additional three women were supplemented in the control group. To confirm the correct use of the test products, the moisturizer group recorded a diary twice daily (in the morning and evening), which was intended to show whether the test product was appropriately applied to the test areas during the test period. By reviewing these diaries at the end of the study, we found that 20 participants in the moisturizer group had not properly adhered to the instructions for using the test product. Thus, data from the remaining 16 participants with sufficient moisturization and 36 participants in the control group were used for further analysis (Table 1).

Objective Clinical Assessments

The severity of skin dryness of the flexor forearm and the anterior lower leg was evaluated before the study (pretest) and after the four-week study period using overall dry skin score (0=absent to 4=severe) by a board-certified dermatologist in Hakodate Central General Hospital.[14]
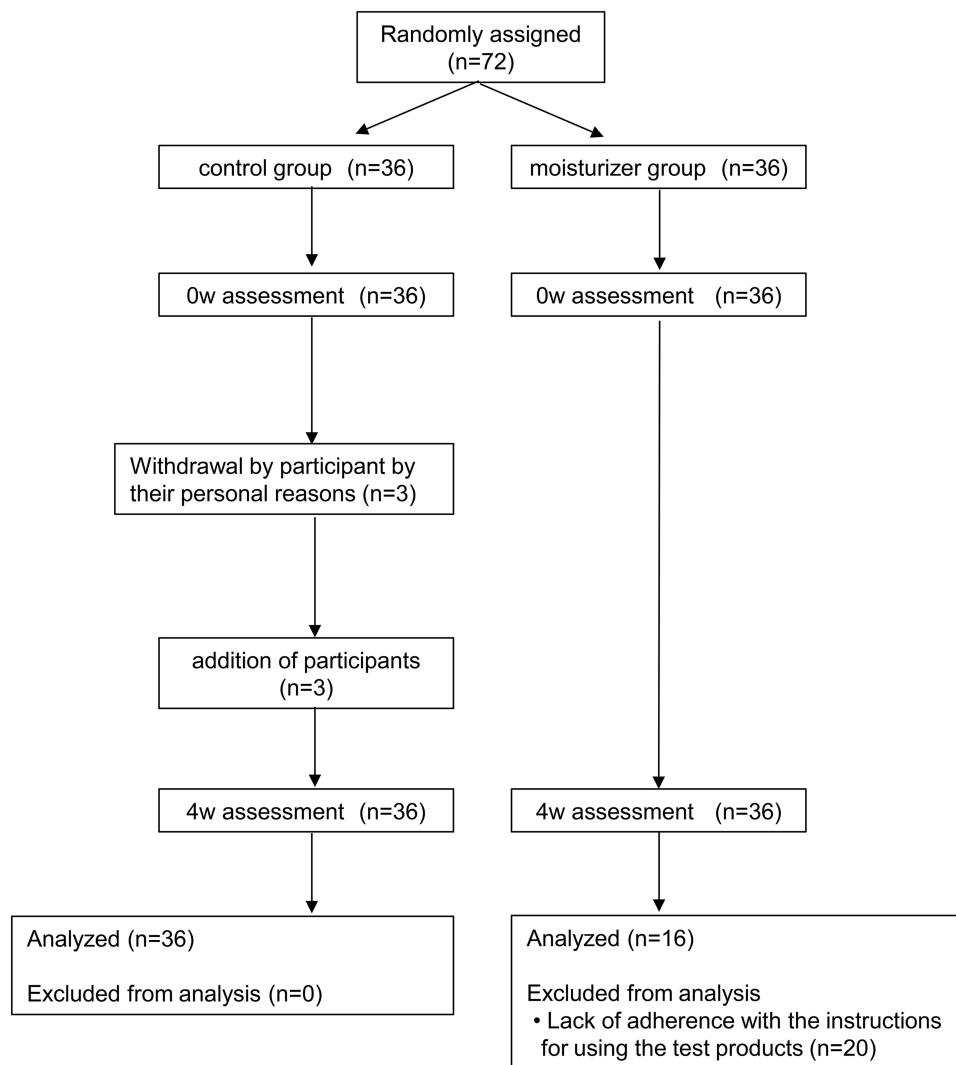
Figure 1 Flow diagram of participants in this study.

Measurement of Epidermal Function

To assess epidermal function, skin hydration (water content of the stratum corneum) and epidermal skin barrier function (transepidermal water loss, TEWL) were measured on the right flexor forearm and anterior lower leg (test area) before the study (pretest) and again after the four-week study period. On each measurement day, the participants had to expose their uncovered test area to standard conditions of room temperature (21 ± 4°C) and humidity (50 ± 8%) before the measurement, to acclimate to the room conditions. The measurement areas were gently wiped with baby wipes, and after approximately 15 min, the water content of the stratum corneum was measured five times using SKICON-200EX (IBS

Table 1 Participant Background

	Number of Participants	Age Range (Median)
Total	52	70–89 (77.0)
Control group	36	70–89 (79.0)
Moisturizer group	16	70–82 (74.5)

Co., Ltd., Hamamatsu, Japan), and the mean value was recorded. TEWL was also measured three times using VAPO SCAN AS-VT100RS (ASCH Japan Co., Ltd., Tokyo, Japan), and the mean value was recorded.

Measurement of Serum Cytokine Levels

Blood samples were collected from all participants before the study (pretest) and after four weeks of the study. Serum levels of seven cytokines (IL-1α, IL-1β, IL-4, IL-5, IL-6, IL-8, and TNF-α) were measured using Human Cytokine/ Chemokine/Growth Factor Panel A Magnetic Bead Panel (Millipore, Billerica, MA, USA).

Questionnaire Survey of the Participants

After four weeks of daily use of the test product, the moisturizer group was instructed to rate their satisfaction for six items, including an overall evaluation of usability and efficacy of the test product on a five-point scale: (1) very dissatisfied, (2) partly dissatisfied, (3) neither satisfied nor dissatisfied, (4) partly satisfied, and (5) very satisfied.

Statistical Analyses

All the data for the overall dry skin scores, water content of the stratum corneum, TEWL, and serum cytokine levels were expressed as means ± standard deviations. Comparisons of the percent changes between the two groups at four weeks were analyzed using Brunner-Munzel test. The correlation of serum cytokine levels with overall dry skin scores, water content of the stratum corneum, TEWL, or questionnaire evaluations were analyzed using Spearman's rank correlation coefficient. $P<0.05$ was considered to be statistically significant.

Results

Questionnaire Evaluation of the Moisturizer

First, in the questionnaire evaluation for the moisturizer group after four-week use, "overall satisfaction" was high enough: all participants responded with "very satisfied" or "partly satisfied" (Figure 2). In addition, regarding questions for usability, including "spreads well" and "favorably compatible with the skin", more than 90% of the participants responded that they were either "very satisfied" or "partly satisfied". Similarly, all participants responded "very satisfied" or "partly satisfied" to the questions related to moisturizing ("moisturizes skin" and "moisturizes skin over time"), suggesting that the participants in the moisturizer group recognized the moisturizing effect. On the other hand, for "favorably compatible with the skin" and "improved skin condition", 6% of participants responded as "neither satisfied nor dissatisfied", although no adverse events were observed during the test period.
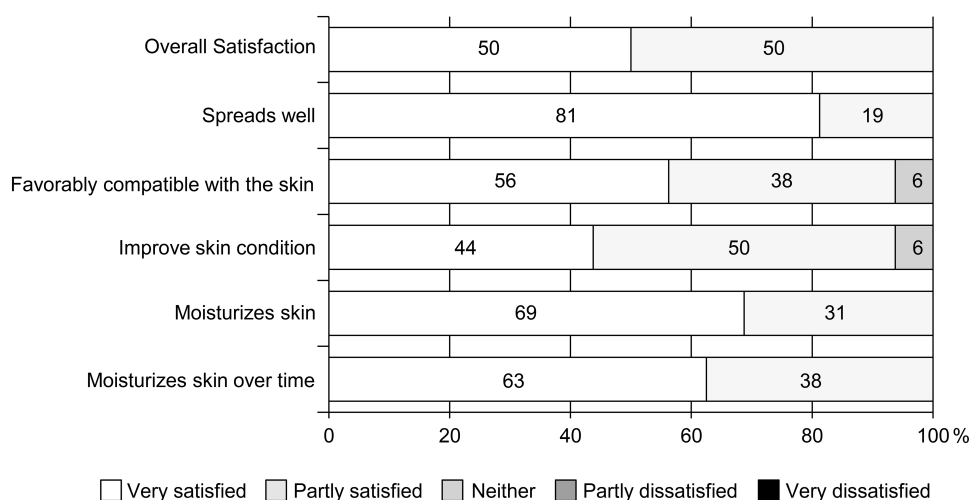

Figure 2 Questionnaire evaluation by the participants in the moisturizer group. The numbers represent percentages.

Changes in Skin Parameters by Moisturizer

Next, to assess the change in skin condition by the use of moisturizer for four weeks, we compared overall dry skin score, water content of the stratum corneum and TEWL between the control group and the moisturizer group. The values at week 0 were set at 100%, and the percent changes in these three parameters at four weeks were evaluated.

As expected, the dry skin severity was reduced in the moisturizer group with statistical significance compared with the control group both in the flexor forearm (89.06 ± 12.81 vs 97.22 ± 7.97, $P<0.05$, Figure 3A) and in the anterior lower leg (76.56 ± 23.22 vs 93.06 ± 22.05, $P<0.01$, Figure 3B). Similarly, the levels of water content of the stratum corneum
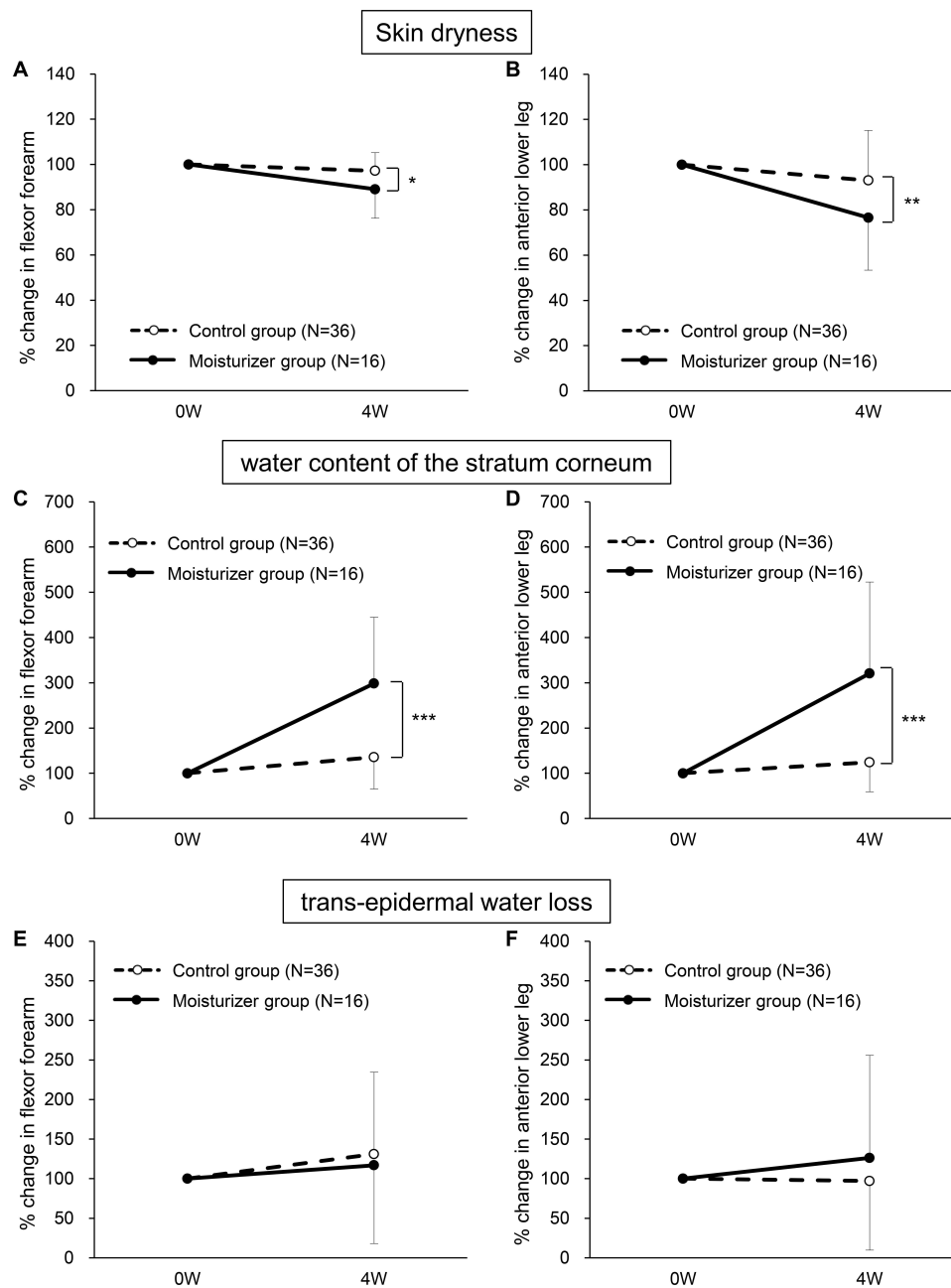

Figure 3 Percent change in skin parameters of flexor forearm and anterior lower leg at four weeks. (**A**) Skin dryness of flexor forearm, (**B**) Skin dryness of anterior lower leg, (**C**) water content of the stratum corneum of flexor forearm, (**D**) water content of the stratum corneum of anterior lower leg, (**E**) transepidermal water loss (TEWL) of flexor forearm, and (**F**) TEWL of anterior lower leg. The values at week 0 were set at 100%. *$P<0.05$,**$P<0.01$, ***$P<0.001$ between the control group and the moisturizer group.

were significantly higher in the moisturizer group both in the flexor forearm (298.66 ± 146.16 vs 135.65 ± 70.28, *P*<0.001, Figure 3C) and in the anterior lower leg (320.80 ± 201.88 vs 124.41 ± 65.26, *P*<0.001, Figure 3D).

On the other hand, there was no significant difference in TEWL between the control group and the moisturizer group in either of the test areas (Figure 3E and F).

Changes in Serum Cytokine Levels by Moisturizer

Serum cytokine levels were then measured to assess changes in circulating inflammatory markers by the use of the moisturizer for four weeks. The cytokine levels below the detection limit at week 0 were excluded from the analysis because the percent changes could not be calculated.

When the percent changes in the serum levels of seven cytokines (IL-1α, IL-1β, IL-4, IL-5, IL-6, IL-8, and TNF-α) after four weeks were compared between the control group and the moisturizer group, only IL-6 level was significantly reduced in the moisturizer group compared with in the control group (42.06 ± 41.24 vs 179.53 ± 431.92, *P*<0.05, Figure 4).

Correlations Between Serum Cytokine Levels and Questionnaire Evaluation

We also analyzed the correlation between changes in serum cytokine levels and questionnaire evaluations after using moisturizer (Table 2), except for IL-1β due to insufficient sample size. We found an inverse correlation between IL-5 and the questionnaire item "moisturizes skin over time" (r_s=−0.616, *P*=0.011), suggesting that the moisturizer improves subjective symptoms of dryness by reducing IL-5 levels.

Correlations Between Serum Cytokine Levels and Skin Parameters

To assess whether changes in objective skin parameters are related to changes in circulating levels of inflammatory cytokines, we analyzed the correlation between skin parameters and serum cytokine levels (Table 3). There was a significant positive correlation of TEWL with IL-4 (r_s=0.535, *P*=0.033).

Correlations Between Serum Cytokine Levels

Lastly, to evaluate the presence of interaction among cytokines, we analyzed the correlation among the seven serum cytokine levels in the moisturizer group (Table 4). There was a positive and significant correlation between IL-5 and IL-6 (r_s=0.807, *P*=0.005), suggesting that they may be regulated by common upstream factors.
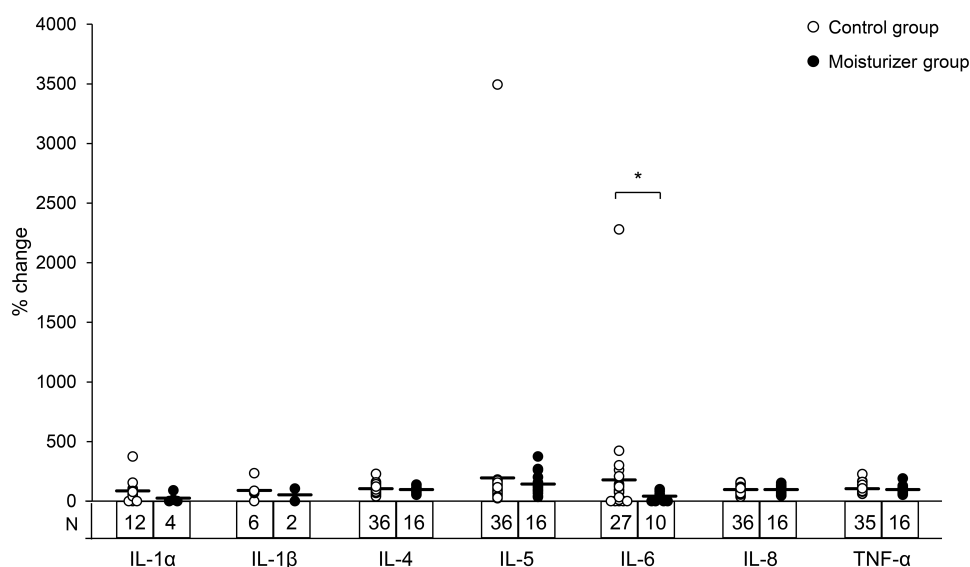

Figure 4 Percent changes in serum cytokine levels at four weeks in the control and moisturizer groups. Bars show means. The significant differences between the control group and the moisturizer group are indicated by **P*<0.05.

Table 2 Correlation Analysis Between Serum Cytokine Levels and Questionnaire Evaluation in Moisturizer Group

			Serum cytokine					
		Number	IL–1α	IL–4	IL–5	IL–6	IL–8	TNF–α
			4	16	16	10	16	16
Questionnaire evaluation	Overall Satisfaction	r_s	0.236	0.271	−0.081	0.287	−0.163	0.027
		p value	0.764	0.310	0.765	0.421	0.547	0.921
	Spreads well	r_s	−0.272	0.191	−0.052	−0.135	−0.122	−0.261
		p value	0.728	0.478	0.848	0.711	0.654	0.330
	Favorably compatible with the skin	r_s	−1.000	0.097	−0.157	−0.229	−0.020	−0.320
		p value	–	0.721	0.561	0.525	0.941	0.227
	Improve skin condition	r_s	0.236	0.470	0.050	0.415	−0.134	0.149
		p value	0.764	0.066	0.856	0.233	0.622	0.583
	Moisturizes skin	r_s	−0.272	0.278	−0.132	0.118	−0.307	−0.366
		p value	0.728	0.297	0.627	0.746	0.247	0.164
	Moisturizes skin over time	r_s	0.236	0.392	−0.616	−0.330	−0.196	−0.028
		p value	0.764	0.133	0.011*	0.352	0.467	0.918

Note: Correlations were assessed by Spearman's Rank Correlation Coefficient (*P < 0.05).

Table 3 Correlation Analysis of Serum Cytokine Levels with Overall Dry Skin Score, Water Content of the Stratum Corneum, or TEWL in Moisturizer Group

			Serum cytokine					
		Number	IL-1α	IL-4	IL-5	IL-6	IL-8	TNF-α
			4	16	16	10	16	16
Overall dry skin score	Flexor forearm	r_s	0.943	0.123	0.232	0.359	−0.014	−0.041
		p value	0.057	0.650	0.387	0.308	0.960	0.880
	Anterior lower leg	r_s	0.889	−0.093	−0.103	0.223	0.336	−0.351
		p value	0.111	0.731	0.705	0.535	0.203	0.183
Water content of the stratum corneum	Flexor forearm	r_s	−0.105	0.088	0.071	−0.331	0.088	0.132
		p value	0.895	0.745	0.795	0.350	0.745	0.625
	Anterior lower leg	r_s	0.316	0.256	−0.085	−0.294	0.029	0.006
		p value	0.684	0.339	0.753	0.410	0.914	0.983
TEWL	Flexor forearm	r_s	−0.211	0.535	−0.103	−0.025	0.171	0.129
		p value	0.789	0.033*	0.704	0.945	0.528	0.633
	Anterior lower leg	r_s	0.316	0.079	−0.265	−0.313	0.021	0.059
		p value	0.684	0.770	0.322	0.379	0.940	0.829

Note: Correlations were assessed by Spearman's Rank Correlation Coefficient (*P < 0.05).

Discussion

The other related study evaluated the changes in plasma levels of three age-related cytokines (IL-1β, IL-6, and TNF-α) and objective skin parameters with and without the use of emollients in 63 elderly people.[11] In the present study, we compared an increased number of circulating cytokine levels (IL-1α, IL-1β, IL-4, IL-5, IL-6, IL-8, and TNF-α) with objective or subjective skin parameters in 75 Japanese women and verified the possibility that the use of moisturizer suppresses systemic inflammation. There were three novel findings.

Table 4 Correlation Analysis Between Serum Cytokine Levels in Moisturizer Group

			Serum cytokine					
			IL–1α	IL–4	IL–5	IL–6	IL–8	TNF–α
Serum cytokine	IL–1α	Number	–	4	4	4	4	4
		r_s	–	0.632	−0.105	0.738	−0.632	−0.105
		p value	–	0.368	0.895	0.262	0.368	0.895
	IL–4	Number	4	–	16	10	16	16
		r_s	0.632	–	0.059	0.306	−0.153	0.303
		p value	0.368	–	0.829	0.389	0.572	0.254
	IL–5	Number	4	16	–	10	16	16
		r_s	−0.105	0.059	–	0.807	0.059	0.491
		p value	0.895	0.829	–	0.005**	0.829	0.053
	IL–6	Number	4	10	10	–	10	10
		r_s	0.738	0.306	0.807	–	0.138	0.469
		p value	0.262	0.389	0.005**	–	0.705	0.172
	IL–8	Number	4	16	16	10	–	16
		r_s	−0.632	−0.153	0.059	0.138	–	0.359
		p value	0.368	0.572	0.829	0.705	–	0.172
	TNF–α	Number	4	16	16	10	16	–
		r_s	−0.105	0.303	0.491	0.469	0.359	–
		p value	0.895	0.254	0.053	0.172	0.172	–

Notes: The correlations were assessed by Spearman's Rank Correlation Coefficient ($**P < 0.01$).

First, we demonstrated changes in subjective skin conditions of elderly people after using the Grafa Moisture Keep Milk MC moisturizer for four weeks: In the questionnaire evaluation, positive evaluation was obtained in the items of subjective moisturizing effect, such as "moisturizes skin" and "moisturizes skin over time". In addition, compared with the control group, the moisturizer group showed a significant decrease in the overall dry skin score and a significant increase in the water content of the stratum corneum. Dry skin was thus suggested to be improved both subjectively and objectively by the use of the moisturizer. On the other hand, consistent with previous reports,[15] there was no significant difference in TEWL levels between the control group and the moisturizer group. Despite the structural and functional deterioration of the barrier function, TEWL values in elderly skin are reported to be lower than those of younger skin, probably due to factors such as an increase in keratinocyte number and a decrease in perspiration.[6] Change of TEWL levels are therefore unlikely to completely reflect the improvement of the barrier function in elderly people.

Next, to evaluate the changes in systemic inflammation status by using the moisturizer, we analyzed cytokine levels in serum. Serum IL-6 level was significantly reduced in the moisturizer group compared with the control group, which is in accordance with the previous study,[11] and supports the hypothesis that skin dysfunction due to aging may be one of the causes of chronic inflammation in elderly people. IL-6 is increased in the blood of patients with various age-related inflammatory diseases, and is thought to be involved in the development of arteriosclerosis and osteoporosis.[16–18] Decrease in circulating IL-6 levels by using moisturizer may therefore lead to the prevention of the onset of these age-related inflammatory diseases and the improvement of their symptoms.

In addition, there was an inverse correlation between serum IL-5 and the subjective moisturizing effect in the questionnaire evaluation, so moisturizer was shown to improve subjective symptoms of dryness by reduction of IL-5 levels. Regarding the relationship between IL-5 and skin barrier function, decrease of E-cadherin in the skin reportedly enhances the production of Type 2 cytokines such as IL-5 by ILC2.[19] Furthermore, there was a positive correlation between IL-5 and IL-6, suggesting that they are regulated by common upstream factors. IL-6 can be directly secreted from epidermal cells upon physical disruption of skin barrier function.[9] Although it cannot be concluded from the results of this study alone, the correlation between IL-5 and IL-6 may indicate that both cytokines are linked to changes in skin barrier function directly or indirectly.

Lastly, we detected a significant positive correlation of TEWL with serum IL-4 levels. IL-4 deficiency has been shown to increase the expression of genes associated with skin barrier function, such as filaggrin and involucrin, and to promote TEWL recovery after skin irritation in murine models.[20]

There are several limitations to this study. First, although this study was conducted in a third-party institution to ensure fairness, objective clinical assessments were performed by only one board-certified dermatologist. Second, IL-1α and IL-1β were also measured in this study, but these cytokine levels in many participants were below the detection limit before using moisturizer. Further studies in subjects with high initial levels of these cytokines are required. Next, in the present study, we aimed to assess the effect of adequate moisturization, so we analyzed only participants who were able to completely apply the moisturizer to the entire body twice a day for four weeks. Because the number of patients determined was limited and insufficient to allow statistically based conclusions, additional studies should be conducted with a larger number of subjects in the future. Additionally, the protocol of moisturizer use, including application range, frequency, and duration needs to be determined to suppress inflammatory cytokines more efficiently.

Conclusion

In summary, not only improved subjective and objective skin condition, but also reduced circulating level of inflammatory cytokine were achieved by applying a moisturizer to the entire body twice a day for four weeks. While many women diligently moisturize one part of the body, such as the face, few moisturize their entire body well every day, and the frequency of moisturizing the body decreases further in elderly people. Future studies are required to prove the hypothesis that the sufficient use of moisturizer may contribute to the prevention and improvement of age-related inflammatory diseases.

Acknowledgments

Disclosure

The author(s) report no conflicts of interest in this work.

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